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Supplementary figure 1. Lineage tracing experiments to investigate the differentiation ability of HPCs

(**a**) Schematic representation showing experimental design of lineage tracing experiments using the *Krt19*^{CreERT}*LSL*^{TdTomato} mice. (**b**) Immunohistochemistry analysis for tdTomato and p21 on injured and uninjured tamoxifen induced *Krt19*^{CreERT}*LSL*^{TdTomato} mice. (**c**) Immunohistochemistry for CYP2D (green), TdTomato (red), and DAPI (blue) on liver of tamoxifen induced *Krt19*^{CreERT}*LSL*^{TdTomato} mice after CDE – recovery. The results shown are representative of 2 experiments with 5-8 mice each group. Scale bars = 50µm.

Supplementary figure 2. Administration of BNF induce hepatocyte damage

(**a**) Morphology by H&E and (**b**) expression of CYP2D6 by isolated purified hepatocytes following liver perfusion and digestion. Arrows denote examples of multinucleated hepatocytes. (**c**) Expression of nuclear p53 following extraction and purification of hepatocytes from AhCre^{+} *Mdm2*$^{flox/flox}$ mice (n = 3) 2 days following induction with 80mg/kg βNF compared to AhCre^{-} *Mdm2*$^{flox/flox}$ controls; arrow highlights low nuclear p53 expression. (**d**) Modified representation of *Mdm2*^{flox} construct outlining primer targets for qPCR assessment of recombination efficiency. (**e**) Serum alkaline phosphatase (**f**) and ALT following induction in with 80mg/kg in AhCre^{+} *Mdm2*$^{flox/flox}$ animals (mean ± s.e.m, Kruskal Wallis Test, n = 3 mice each group, except day 8 where n=1 mouse due to cohort morbidity). (**g**) Expression of apoptosis associated p53-dependent gene *Bax* in whole liver over time following induction with 80mg/kg βNF in AhCre^{+} *Mdm2*$^{flox/flox}$ mice (mean ± s.e.m, One-way ANOVA.; n=3 mice each control time point and n = 3,3,6,5 for experimental time points). (**h**) Immunohistochemistry for lactate dehydrogenase of healthy mice, βNF induced AhCre^{-} *Mdm2*$^{flox/flox}$ controls and βNF induced AhCre^{+} *Mdm2*$^{flox/flox}$ mice. Representative images shown are representative of 2 experiments with 12 mice in total. Scale bars = 50 μm

Supplementary figure 3. Activation of ductular reaction following hepatocyte damage

Detection of (**a**) EpCAM (**b**) DLK1 (**c**) A6 (inset, $AhCre^-$ control) expressing cells following Δ*Mdm2* in hepatocytes. (**d**) Immunohistochemistry for CD24 (red), EpCAM (green), DAPI (blue) on CDE treated and βNF induced $AhCre^+Mdm2^{flox/flox}$ mice. Representative images are shown are representative of 3 experiments with 5-8 mice each group (**e**) *Ck19* expression in the whole liver of the induced $Mdm2^{flox/flox}$ mice versus uninduced control over time (mean ± s.e.m, One-way ANOVA with Bonferroni correction. P=0.0058 day 8; n=3 mice each control time point and n = 3,3,6,5 for experimental time points, repeated twice). (**f**) *Ascl2* expression of $Mdm2^{flox/flox}$ mice over time following induction (mean ± s.e.m, One-way ANOVA with Bonferroni correction. P=0.05; n=3 mice, repeated twice). (**g**) Quantitative comparison of the panCK positive cells between the uninduced control, $Mdm2^{flox/-}$ $Mdm2^{flox/flox}$, and the choline deficient ethionine supplemented diet (CDE) model (mean ± s.e.m, One-way ANOVA with Bonferroni correction. n = 4,6,5,5 mice each group respectively). (**h**) BrdU and panCK co-expressing cells can be observed 2 days after △*Mdm2*. Representative images are shown are representative of 3 mice each group. Scale bars = 50 μm.

Supplementary figure 4. Expandability of EpCAM+ CD24+ CD133+ population *in vitro*

(**a**) Morphology of cdHPCs after passaging with trypsin (left) or diluted trypsin (right). Insets show high magnification pictures. (**b**) Percentage of total EpCAM+CD24+CD133+ cells after *in vitro* expansion. (mean ± s.e.m, n = 6 biological replicates, Mann-Whitney test) (**c**) mRNA expression in relative to housekeeping gene (*Ppia)* of expanded cdHPCs (mean ± s.e.m, n= 3 biological replicates). (**d**) Heat map representation comparing mRNA expression in relative to housekeeping gene *Ppia* of cdHPC clones and primary hepatocytes. (**e**) Relative mRNA expression for HPC related genes *Lgr5, EpCAM, Albumin, Ck19, Spp1, Sox9* on early and late passages cdHPCs (mean ± s.e.m, Kruskal Wallis test. *P* >0.05, except *Lgr5 P*=0.0286; n=4 biological replicates). (**f**) Immunocytochemistry for HPC markers Sox9, CK19, OPN, and HNF1β on early and late passages cdHPC. (**g**) Cell area, roundness, and width to length ratio of early and late passages cdHPCs (mean ± s.e.m, Kruskal Wallis test *P*= 0.0107; n=4 biological replicates). (**h**) Calculation for the average number of cell division after 10 passages; n=3 biological replicates. Representative images represent data obtained form 3 individual experiments. Scale Bar = 100 µm

Supplementary figure 5. Ability to differentiate towards both hepatic and biliary lineage i*n vitro*

(**a**) FACS analysis of LGR5, CD31 and CD45 expression on *in vitro* expanded cdHPCs. Isotype control (blue line) (**b**) Immunocytochemistry for desmin and GFAP in *in vitro* expanded HPCs. (isolated stellate cells as positive control). (**c**) *In vitro* differentiation of expanded HPCs into cholangiocytes stained with activated bile duct marker MIC1C3 (green) and Hnf1β (red), DAPI (blue). (**d**) *In vitro* differentiation of expanded HPCs into hepatocytes dotted line demarcates a hepatocyte like colony, upper panel. Increase Glycogen storage detected by Periodic acid-Schiff staining on differentiated cdHPCs. (**e**) *Alb* mRNA expression and secreted protein following hepatocyte differentiation (mean ± s.e.m, P = 0.007 Mann-Whitney test; n=5 biological replicates). Lower histograms demonstrate expression of cholangiocyte related genes, and hepatocyte transcription factor *Hnf1α*. Representative images are shown as representative of 3 individual experiments. Scale Bars = 50 μm

Supplementary figure 6. Secondary clone sorting assay for the *in vitro* expanded HPCs.

(**a**) EpCAM and CD24 expression of *in vitro* expanded HPCs. (**b**) EpCAM and CD24 expression analysis of secondary clones 7 days after replating. (**c**) Percentage of EpCAM+ $CD24^{Hi}$ population in secondary clone cultures (mean ± s.e.m, Kruskal Wallis test; $P = 0.0076$). Data are represented as mean ± s.e.m., n=5 biological replicates. Representative images represents 3 individual experiments.

Supplementary figure 7. Liver repopulating capacity of the *in vitro* expanded HPCs.

(**a**) Stitched image of GFP expressing cells in CAG-GFP HPCs transplanted animals. (**b**) Detection of GFP, ductular marker (panCK) and hepatocyte marker (HNF4α) in transplanted animals and non-transplanted controls (white arrow, GFP- panCK+; red arrow, GFP+ panCK+; green arrow, GFP+ HNF4α-; yellow arrow, GFP+ HNF4α+). (**c**) Detection of GFP and proliferation marker (Ki67) or senescence marker (p21) in transplanted animals and non-transplanted controls (insets, higher magnification) three months after HPC transplantation (upper panel). (White arrows show GFP+ Ki67+ hepatocytes; Yellow arrow shows p21- GFP+ hepatocytes). Data shown here are representative of 3 experiments with 8-10 mice each group. Scale Bars = 50 μm, except stitched image (**a**) where scale bar = 200μm.

Supplementary table 1: Recombination efficiency of the *ΔMdm* system and summary of experimental design

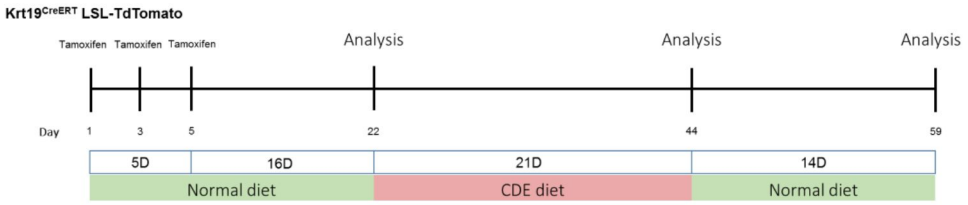
Efficiency of recombination as assessed by over-expression of nuclear p53 in hepatocytes; n≥3 each group († by qPCR of genomic DNA from purified *ex vivo* hepatocytes; see also **Supplementary Fig 1**). No up-regulation of hepatocellular p53 was seen in uninduced AhCre^{+} *Mdm2*$^{flox/flox}$ or AhCre^{-} *Mdm2*$^{flox/+}$ control mice given 80mg/Kg βNF. Mice induced with 240mg/kg βNF were given 3 separate injections of 80mg/Kg βNF over 24hrs. Schematic representation of experimental design for the *Ah*Cre*ΔMdm2* mice.

Supplementary table 2: List of antibodies used

Antibodies used for antigen detection in the current study are provided together with mode of tissue fixation, method of antigen retrieval and working dilution. Formalin = fixation for 6 hours in 10% formalin in PBS, NaC = 100mM Sodium Citrate pH 6.0, and Tris EDTA = 100mM Tris EDTA pH 9.0.

Supplementary 1

a



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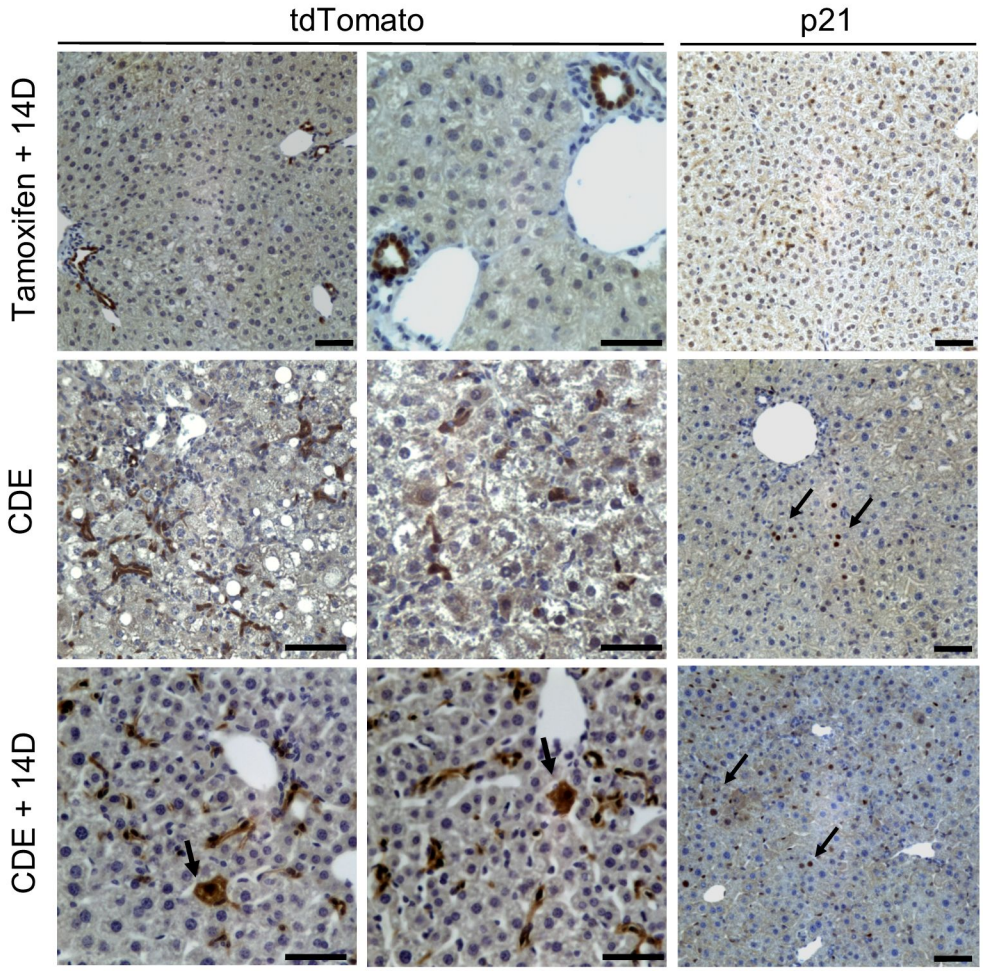

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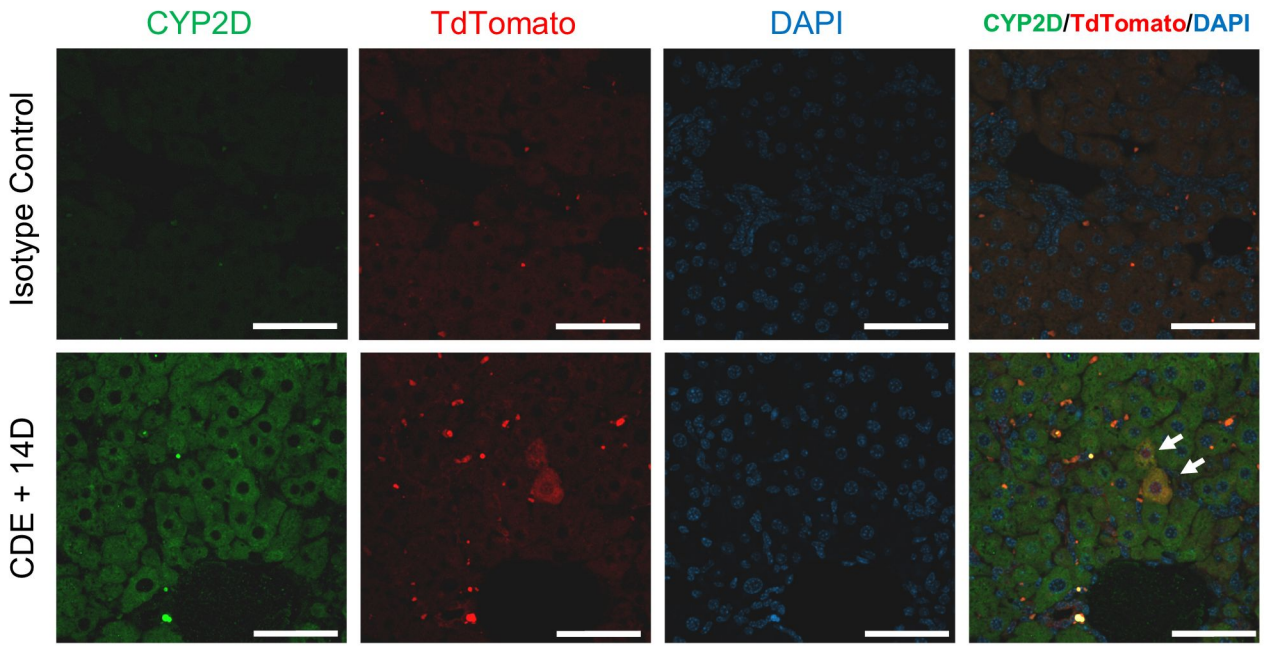

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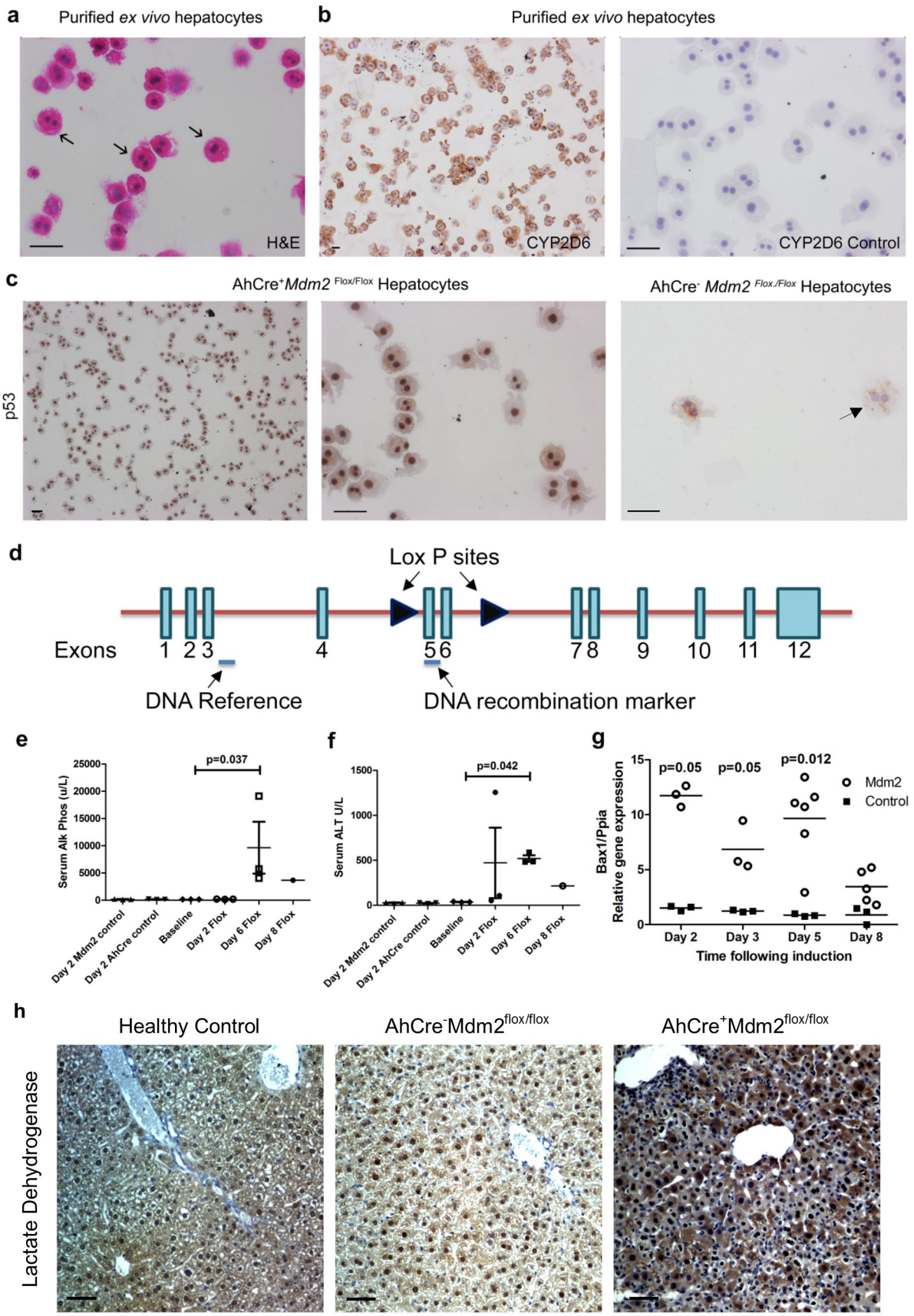

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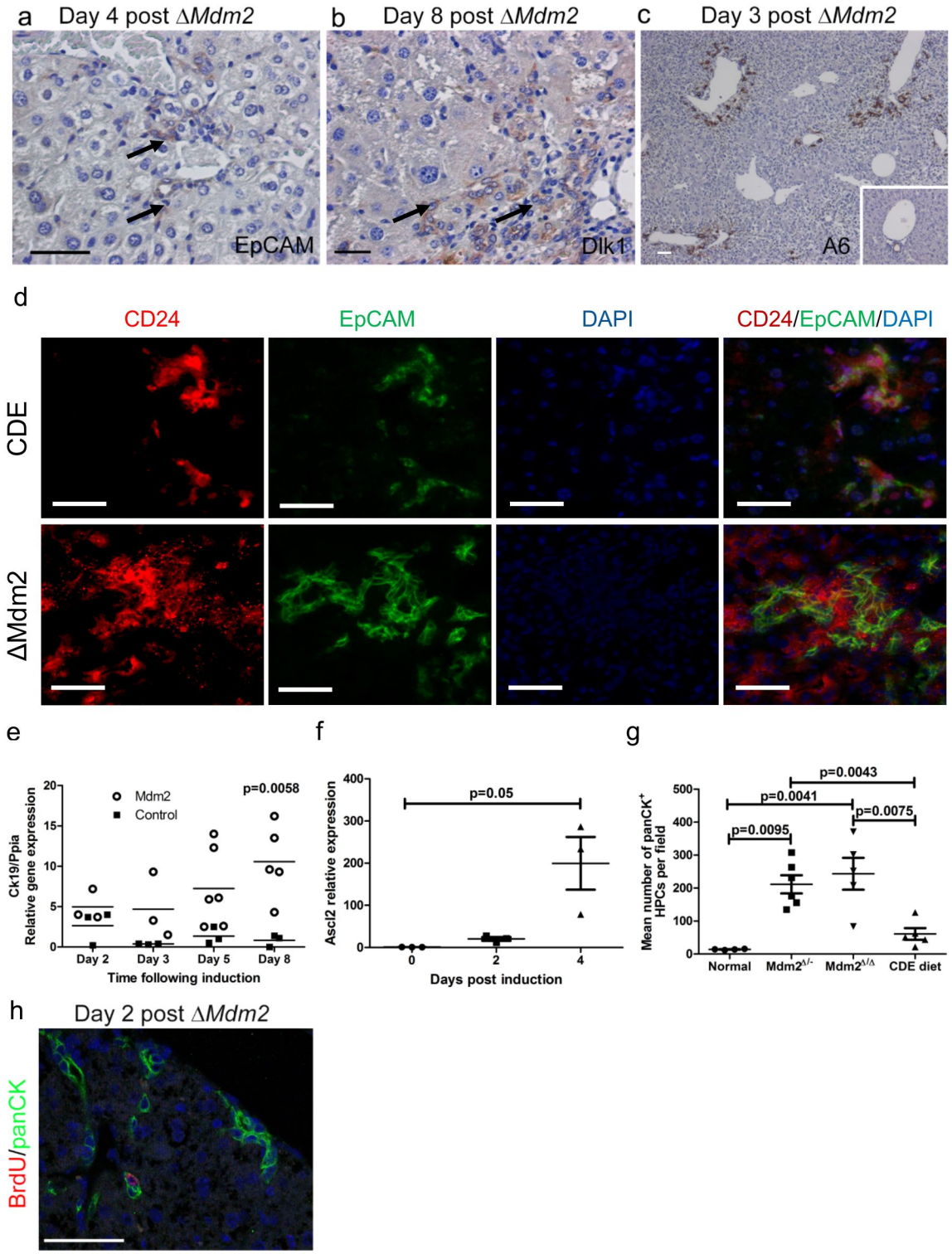

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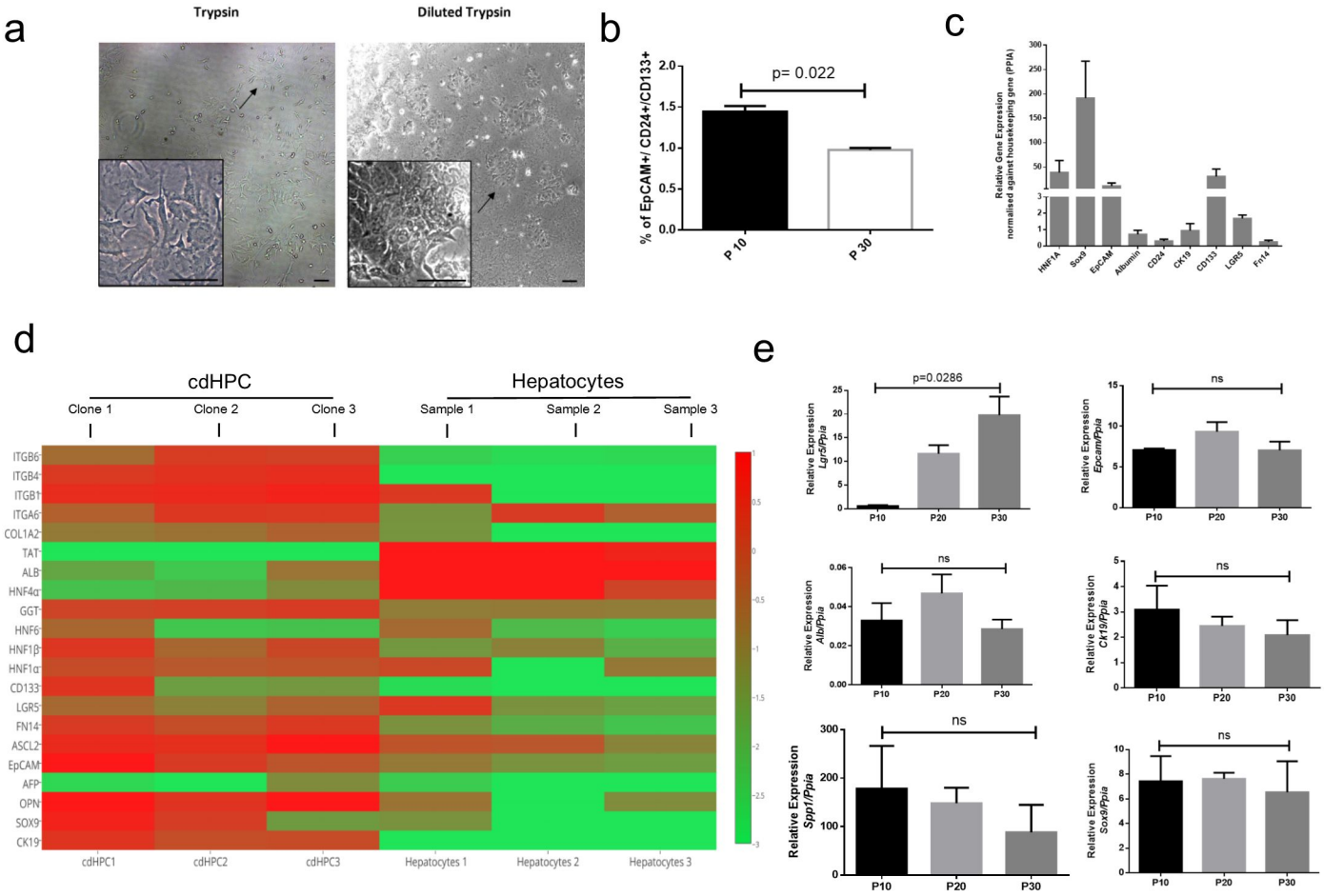
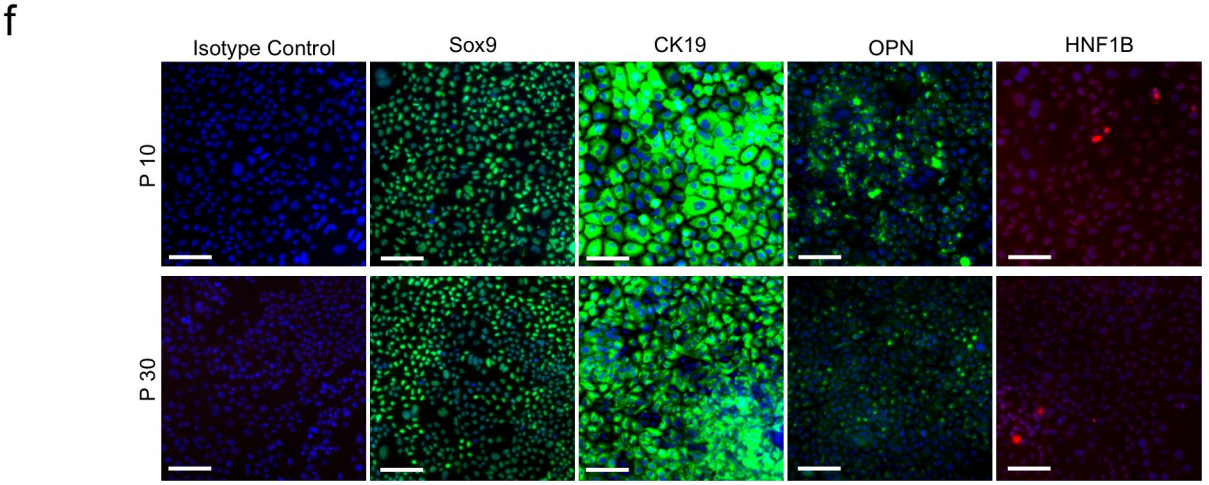
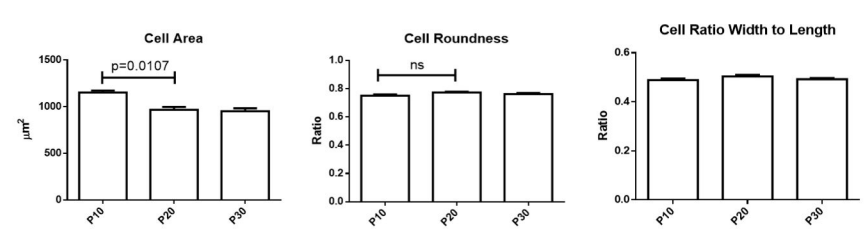






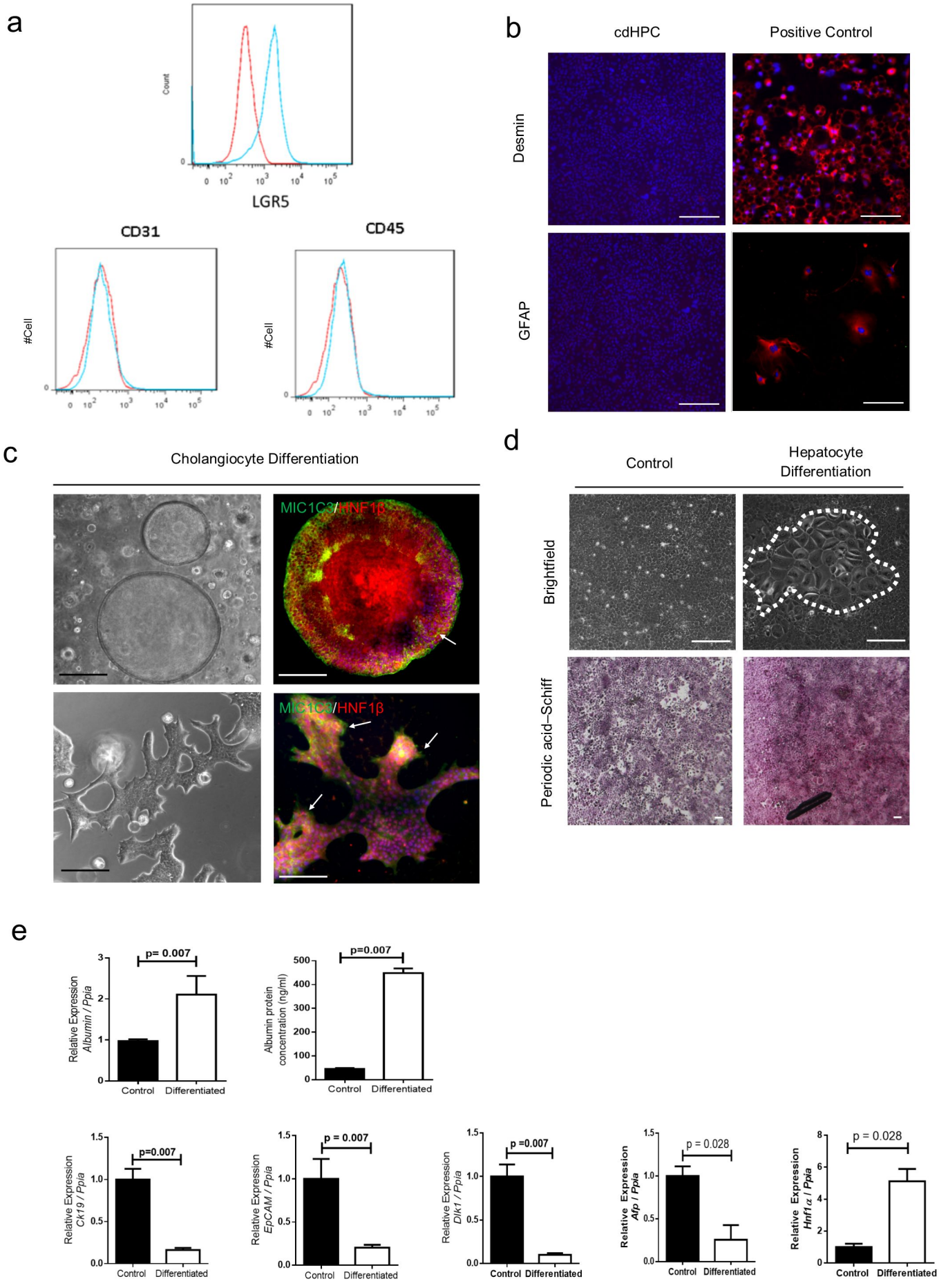
h

Nt : Number of cells at P10 (1.7×10^{12})
N0: Initial number of cells (500)
n: number of division

Expandability of cdHPCs

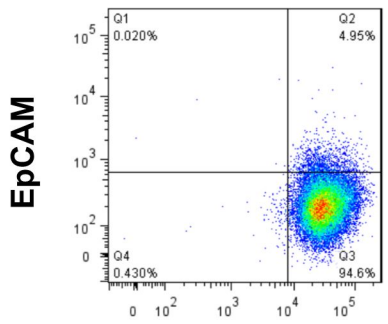
$Nt = N0\ 2^n$
$(1.7 \times 10^{12}) = (500)2^n$
$N = 31.7$

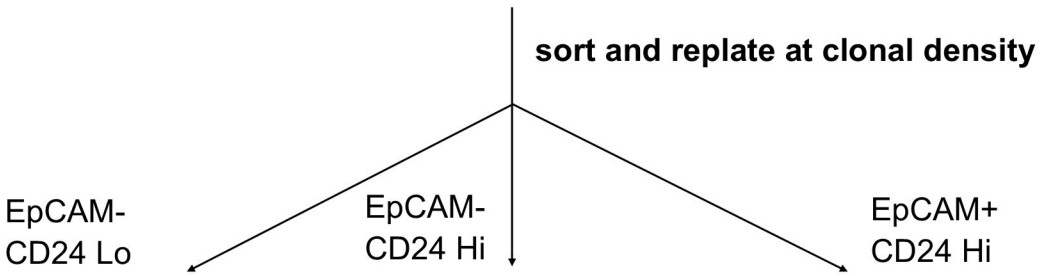
Supplementary 5



Supplementary 6

a





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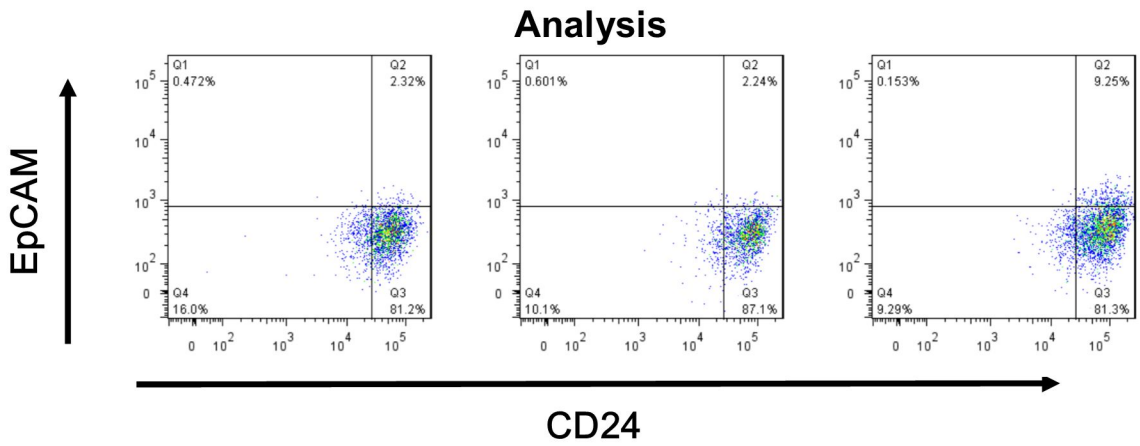

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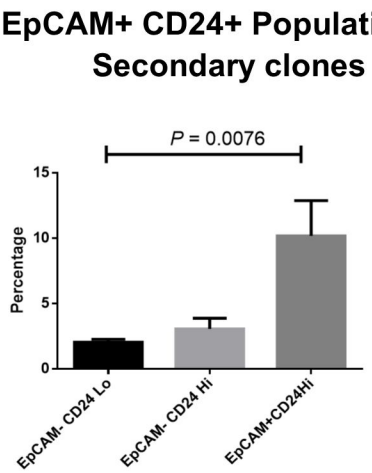

Supplementary 7